

SUPPLEMENTAL DATA

Table 1. Proteins identified by tandem mass spectrometry of native gel-purified RNA-protein complexes. All proteins that were identified in the gel-purified complex shown in Figure 1D (Preparation 1) and in the native gel-purified complex obtained by an alternate chromatography scheme (Preparation 2; see experimental procedures) are listed. Numbers represent the % amino acid sequence coverage, with the number of unique peptides in parentheses. The annotated function is from the NCBI database.

	Protein	Prep 1	Prep 2	Annotated Function
Cas proteins	PF1129	56.0 (54)	26.9 (20)	hypothetical protein PF1129
	PF1128	40.4 (13)	20.5 (5)	hypothetical protein PF1128
	PF1126	58.3 (14)	17.6 (3)	hypothetical protein PF1126
	PF1124	16.2 (5)	11.8 (4)	hypothetical protein PF1124
	PF0352	28.1 (5)		hypothetical protein PF0352
	PF1125	24.9 (5)		hypothetical protein PF1125
	PF1130	3.6 (1)		hypothetical protein PF1130
Non-Cas proteins	PF1717	76.2 (28)		translation initiation factor IF-2 gamma subunit
	PF1683	73.6 (19)		N-acetyl-gamma-glutamyl-phosphate reductase
	PF0990	60.6 (26)		phenylalanyl-tRNA synthetase beta subunit
	PF1685	59.0 (20)		acetylornithine/acetyl-lysine aminotransferase
	PF0481	55.7 (7)		translation initiation factor IF-2 beta subunit
	PF1827	53.8 (14)	20.8 (4)	hypothetical protein PF1827
	PF1881	51.6 (4)		chromatin protein
	PF0989	45.1 (22)		phenylalanyl-tRNA synthetase alpha subunit
	PF0124	34.3 (14)		hypothetical protein PF0124
	PF1140	30.2 (7)		translation initiation factor IF-2 alpha subunit
	PF0495	29.7 (34)		reverse gyrase
	PF1204	29.2 (11)		seryl-tRNA synthetase
	PF1264	26.1 (3)		translation initiation factor IF-5A
	PF0351	25.6 (8)		hypothetical protein PF0351
	PF1238	23.9 (14)		putative ABC transporter
	PF1615	23.1 (18)		hypothetical protein PF1615
	PF0496	21.2 (5)		hypothetical protein PF0496
	PF0594	18.4 (4)	14.3 (2)	Ornithine carbamoyltransferase
	PF1405	16.6 (10)	12.9 (7)	Cleavage and polyadenylation specificity factor protein
	PF0547	15.8 (5)		hypothetical protein PF0547
	PF0969	14.7 (4)		2-ketovalerate ferredoxin oxidoreductase subunit alpha
	PF0220	14.1 (6)	13.6 (7)	Hexulose-6-phosphate synthase
	PF1375	13.3 (6)		elongation factor Tu
	PF1976	12.1 (6)		L-aspartate oxidase
	PF0666	11.5 (6)		nol1-nop2-sun family putative nucleolar protein IV
	PF1746	11.0 (6)		hypothetical protein PF1746
	PF0251	11.0 (4)		hypothetical protein PF0251
	PF1579	10.4 (7)		DNA topoisomerase VI subunit B

PF0966	10.4 (4)		2-oxoglutarate ferredoxin oxidoreductase
PF0533	10.1 (7)		indolepyruvate ferredoxin oxidoreductase subunit a
PF1578	9.2 (3)		DNA topoisomerase VI subunit A
PF0026	8.8 (4)		tRNA nucleotidyltransferase
PF1540	8.7 (5)		ADP forming acetyl coenzyme A synthetase
PF1203	8.1 (5)		formaldehyde:ferredoxin oxidoreductase
PF1046	7.9 (3)		queuine tRNA-ribosyltransferase
PF0464	7.5 (4)		glyceraldehyde-3-phosphate:ferredoxin oxidoreductase
PF1768	5.1 (2)		2-oxoglutarate ferredoxin oxidoreductase
PF0440	3.9 (5)		ribonucleotide-diphosphate reductase alpha subunit
PF1843	1.7 (2)	7.3 (6)	chromosome segregation protein smc
PF0102		76.6 (15)	hypothetical protein PF0102
PF1883		74.9 (13)	small heat shock protein
PF1548		63.3 (24)	hypothetical protein PF1548
PF1931		27.9 (6)	hypothetical protein PF1931
PF0162		11.2 (2)	hypothetical protein PF0162
PF0204		6.0 (2)	hypothetical protein PF0204
PF1871		3.7 (1)	"N(2),N(2)-dimethylguanosine tRNA methyltransferase"
PF1245		2.2 (1)	hypothetical d-nopaline dehydrogenase
PF1167		1.5 (1)	chromosome segregation protein

Table 2. Cloned psiRNAs. The sequences of the CRISPR-derived clones obtained from the upper and lower psiRNA bands (Figure 2A) are shown. The length and psiRNA designation of each sequence is indicated. (Some CRISPR loci encode indistinguishable psiRNAs.) Repeat sequence is shown in bold and the sequences are aligned by the 5' repeat sequences. The assignment of sequences (TT) shaded in grey (and corresponding clone lengths) is ambiguous due to the addition of polyA in the cloning procedure. One rRNA fragment was also cloned (not shown).

	psiRNA sequence	length	psiRNA
Upper Band	ATTGAAAGTTAGCAAATTGCCGATTATTGCACATAAAAAAAATAG	45	1.14
	ATTGAAAGTTAGCAAATTGCCGATTATTGCACATAAAAAAAATAG	45	1.14
	ATTGAAAGACTGGATTGAGAGCAACTTGTGAATTATGTCGCAA	45	1.40
	AATTGAAAGTGTTCATCAGCACTTCTTCTGACTCTGCTCC	43	2.01
	AATTGAAAGTGTTCATCGCACTTCTTCTGACTCTGCTCC	42	2.01
	ATTGAAAGCTAATTACGCTTAGCTCGTGTCAACCCCTAAC	43	2.19
	ATTGAAAGCTAATTACGCTTAGCTCGTGTCAACCC	38	2.19
	ATTGAAAGTTGAGTTGAAGGCCACTCTTGAAGCCTATCAGAGT	45	4.02
	ATTGAAAGGCTTCAGGTCTCAATATTCAATCCCAGTCCCTTC	45	4.03
	ATTGAAAGGCTTCAGGTCTCAATATTCAATCCCAGTCCCTTC	45	4.03
	TTGAAAGTCTCACCTTACAAGCTCTGAATCTATCGAATT	44	5.09
	TTGAAAGGTACGTAATTGCCAAGTTCTTGGATACCCTTC	43	5.12
	ATTGAAAGGTGGATAATATAATCCCTGTTTCCCAAAGA	39	5.13
	ATTGAAAGTGGAACTCTATCAAGGTTGCAACACCTGCTCCGC	45	5.24
	TTGAAAGGACAAAGAACCTCCCTAGCGTCCCTCCCCGTGA	38	6.07
	ATTGAAAGTGGGTCTCGTCGAATCGGTGCAGTATTCTAACGCC	45	6.26
	ATTGAAAGATCTCATACCAATGCTGCAAAATCAATTG	45	6.40
	ATTGAAAGTAAACTTAAGCTGGGATGGGCTATATACAAAGACAGA	45	6.42
	AATTGAAAGTCAAGAGTTCTATCCTGCTTCACAACACCCATATAA	46	7.11
	ATTGAAAGGCGTTAATGAACAATAAGCCTGACACGAACATAAA	43	1.06, 2.02
Bottom Band	ATTGAAAGCCGGTTCTGCACCCGAAACTTCATACCAA	39	1.03
	AATTGAAAGCCGGTTCTGCACCCGAAACTTCATACCAA	39	1.03
	ATTGAAAGGTAGTGAGGCGTTGAACCTGACCCACCA	39	1.08
	ATTGAAAGTGAGTTGTTAGTCTAACCTTACACCAC	38	1.19
	ATTGAAAGTGCCTATTCTCGGGTCAAGCCTCCCAGCCT	39	1.22
	TTGAAAGCACCACGATGAAGGTACCGTTCAAC	37	1.37
	TTGAAAGCACCACGATGAAGGTACCGTTCAAC	37	1.37
	ATTGAAAGTGTTCATCGCACTTCTTCTGAC	33	2.01
	ATTGAAAGCTTCTCGAACGCTAGTTAGTGTGTCAAG	39	2.05
	ATTGAAAGTTCTAGAACGTTCTTGTGAGAGCCAGGAGC	39	2.06
	TTGAAAGCTAATTATGCTTAGCTCGTGTCAACCC	39	2.19
	TTGAAAGCTAATTACGCTTAGCTCGTGTCAAC	36	2.19
	AGGAATGTTGCTCAATGCAAAGGCTCACCGCT	33	4.01
	AAAGTCTCAATTGGGGAGTGCTTAATGGCTTT	34	4.12
	ATTGAAAGGAACTCCTCGATTTAGTACCTGTGTC	36	5.05
	ATTGAAAGGCCACATAAGACATTGTCATACAAAGTAGG	37	5.11

ATTGAAAGGTACGTAATCGCCAAGTCCTTGGAGA	38	5.12
ATTGAAAGGTGGATAATATAATCCCTGTTTCCCAAGA	39	5.13
ATTGAAAGCAGTTCTACTTGATAAGACTGTGGTGGTTA	39	6.03
ATTGAAAGGACAAAGAACTCCCTAGCGTCCCTCCCCGTG	39	6.07
AATTGAAAGTTCTGCCGTCCCTTCTGACGAACCTCAT	39	6.09
ATTGAAAGGCACCTCTTCACCATGCCGTCTGGATTGC	39	6.14
AGTTGTAGGCTCGTGGACTTGGCTTCCACACAACTA	36	6.24
ATTGAAAGTATCTATTGTACAGGTACTTGTACACGT	37	7.14
ATTGAA GCTTGCCAACCTCTCTAGAAACGCCAC	35	8.06
ATTGAAAGGCCTTAATGAACAATAAGCCTGACACGAAC	38	1.06, 2.02
ATTGAAAGTAATCTCAATAACTTGGCTTTCTGTG	39	4.14, 5.19
ATTGAAAGTAATCTCAATAACTTGGCTTTCTGTG	39	4.14, 5.19

Supplemental Figure 1. Characterization of the activity of the psiRNA-Cmr protein complex. **A)** Determination of the end groups of the cleavage products generated by the psiRNA-Cmr complex. 5' end-labeled target RNA (- psiRNPs) and psiRNA-Cmr complex cleavage products (+ psiRNPs) were subject to polyadenylation by *E. coli* polyA polymerase (+) or no treatment (-). The position of the target RNA and polyadenylated target RNA are indicated. Arrows indicate the locations of the cleavage products. No polyadenylation of the cleavage products was observed, indicating the lack of 3' hydroxy groups on these RNAs. **B)** Divalent metal dependence of the psiRNA-Cmr complex. Cleavage reactions were performed in the presence of 0.1 mM EDTA and 1 mM of the indicated divalent metals or no metal (-). The cleavage products are indicated by arrows.

